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Adhering to Mainstream Concepts Homeopathic Therapy Explained as Protein-based Antigen-specific Immunotherapy Backed by Non-specific Immunotherapy

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Abstract The apparent absence of drugs in ultra-diluted homeopathic medicines and contested clinical trial results plague homeopathy. Here it is argued that other than drugs, homeopathic medicines contain proteins as components of microbial lysates (products of lysis or disintegration of microbial cells), given that ubiquitous microorganisms from the surrounding environment are unknowingly and unavoidably incorporated into the homeopathic medicines during their preparation and are killed and lysed in ethanol-water drug vehicle forming immunomodulatory microbial lysates during 'potentization' (dilution and vigorous shaking) of the medicines. The drugs present in the homeopathic medicines bind to the proteins, which are the major ingredients of the microbial lysates. The drug-protein interaction modulates the conformations and in effect, the immunogenicity of the proteins (designated as modulated proteins). In ultra-diluted medicines even in the absence of drugs, unmodulated proteins are modulated through interactions with allosterically coupled modulated proteins (protein-protein interaction). The modulated proteins of characteristic immunogenicity present in the homeopathic medicines mediate antigen-specific mucosal (sublingual) immunotherapy like vaccine therapy via 'similia principle'. In addition, immunomodulatory microbial lysates present in the homeopathic medicines mediate non-specific immunotherapy and also provide adjuvants for antigen-specific immunotherapy. The proposed hypothesis without invoking any controversial concept can explain the basic 'laws' of homeopathy. Incidentally, immunomodulatory activities of homeopathic medicines reported by different workers support the hypothesis. As immunotherapy in homeopathy is accidental and hence, in crude form, clinical trial results may occasionally show inconsistencies. However, probing and refining homeopathy from the perspective of immunotherapy may bring forth a simple, reliable and affordable immunotherapy for various diseases.

Keywords Immunotherapy, Homeopathy, Proteins, Immunomodulators, Microbial Lysates, Bacterial Lysates

1. Introduction

Homeopathy is vexed by two major issues that elude resolution. First, in order to understand the 'mystery' behind the medicinal effects of ultra-dilutions lacking in drugs (dilutions beyond the so-called 'Avogadro's limit', i.e., dilution 12C (1 in 10^{24}) and above), different mechanisms [1,2] based on persistent memory of water, nanoparticles, weak quantum theory, quantum coherence

domains, drug-imprinted impurities, water-ethanol clusters/clathrates etc. have been proposed, which are yet to gain mainstream acceptance. Second, although some clinical trials and meta-analyses [3-6] upheld the therapeutic effects of homeopathic medicines above placebo, other studies (including a few government-sponsored ones) could not corroborate the positive results [7, 8], and the controversy remains.

In the present paper, it is proposed that other than drugs, homeopathic medicines contain microbial lysates (having proteins as major ingredients) and homeopathic therapy, by accident rather than by design, is a protein-based antigen-specific sublingual immunotherapy (albeit, in rudimentary form) augmented by non-specific immunotherapy mediated by immunomodulatory microbial lysates. The hypothesis can explain the basic tenets of homeopathy without resorting to any controversial theory. Also, the proposition can account for the medicinal properties of ultra-dilutions devoid of drugs and contested clinical trial results.

2. How are Immunomodulatory Microbial Lysates Containing Proteins Formed in Homeopathic Medicines?

Bioaerosols comprising bacteria, fungi and viruses are ubiquitous in the atmosphere (concentration ~ 10^5 m^{-3}) [9, 10]. Accordingly, homeopathic medicines like other pharmaceutical products are prepared in pharma cleanrooms. However, even in high-end cleanrooms, microbes are found from air, water (including distilled water [11]), raw materials, cleanroom facilities and above all, cleanroom personnel, who shed a lot of microorganisms [12]. Pharmaceutical product recalls (both allopathic [13] and homeopathic [14]) owing to contamination by pathogenic microbes are not uncommon even in recent years. Also, some homeopathic mother tinctures have been found to be contaminated with pathogenic microorganisms [15]. Incidentally, given that about 97-98% of environmental microbes are nonpathogenic [9, 10], pharmaceutical products are expected to contain a lot more nonpathogenic microorganisms as contaminants, though they are of little practical consequence and are also difficult to detect as most of the environmental nonpathogenic microbes are nonculturable [16]. Hence, during preparation of homeopathic medicines ambient microbes are unknowingly and unavoidably introduced in the homeopathic medicines and are killed and lysed by chemical disintegration (fluidization and disruption of microbial cell membranes in ethanol-water homeopathic drug vehicle) [17, 18] or by mechanical disintegration (vigorous shaking or succussion during potentization) [17] or by the combined effect of chemical and mechanical disintegration forming microbial lysates in the medicines. Interestingly, microbial lysates can also form in the Fincke’s fluxion method (now obsolete) of potentization done in plain water without succussion [19], where turbulence generated in the process [19] may lead to cavitation [20] and disintegration of microbial cell walls. Hence, microbial lysates comprising proteins (major ingredients), protein fragments (peptides), nucleic acids, polysaccharides, peptidoglycans etc. [17, 21, 22] should be present in homeopathic medicines along with externally added drugs. Most importantly, proteins present in the microbial lysates are not degraded (i.e., primary structure of the proteins not disrupted and immunogenicity not lost) but may be fully or partially denatured (unfolded) retaining their immunogenicity [21, 23].

3. How do Homeopathic Medicines Containing Proteins Mediate Antigen-specific Immunotherapy Like Vaccine Therapy via 'Law of Similars'?

Immunotherapy is a mode of treatment where the immune system is manipulated to boost or suppress the immunity so as to maintain homeostasis and in antigen-specific immunotherapy the immunotherapy is directed towards a specific (or closely associated) antigen(s) [24, 25]. Proteins can be potent immunogens (antigens that can induce an immune response) and are promising candidates for mucosal immunotherapy [21, 26]. Incidentally, a wide variety of ligands such as nucleic acids, peptides, ions, metals, and solvents can bind to proteins and the ligand binding modulates the conformations and therefore, the immunogenicity of the proteins [27-29]. Accordingly, drugs present in the homeopathic medicines bind to the proteins and modulate their conformations (designated as modulated proteins) and in effect, their immunogenicity. Consequently, a diverse set of homeopathic medicines containing modulated proteins of characteristic immunogenicity is obtained, which mediates antigen-specific immunotherapy that is patient-specific via ‘law of similars’ as described below.

According to the ‘law of similars’ if the ‘drug proving’ symptoms (also called ‘drug picture’) of a homeopathic medicine matches the symptoms of a patient, the very medicine is supposed to cure the patient [1, 2]. The ‘drug proving’ symptoms of a homeopathic medicine are a specific set of symptoms experienced by healthy volunteers after administration of multiple doses of the medicine in accordance with the standard procedure of ‘drug proving’ [30]. The symptoms observed in healthy volunteers during ‘drug-proving’ can be understood from the proposed hypothesis as the volunteers’ immune response (may be manifested as physical, emotional and/or behavioural symptoms as immune reaction is also linked to neural, psychological and endocrine functions [31]) to the antigenic challenge with the specific homeopathic medicine containing modulate proteins of characteristic

immunogenicity as the antigens (immunogens). On the other hand, a patient's symptoms are the immune response to the (antigenic) challenge with the antigens of the pathogen that has infected the patient. A similarity between the 'drug proving' symptoms and the patient's symptoms should indicate a similarity or functional equivalence between the antigens present in the specific homeopathic medicine as modulated proteins of characteristic immunogenicity and the antigens present on the surface of the pathogen. According to the 'law of similars', the administration of the specific medicine (patient-specific medicine) to the patient is expected to eradicate the symptoms and cure the patient.

Apparently, the rationale behind the 'law of similars' seems counterintuitive as an administration of antigens, similar to the antigens of a pathogen, to a patient who is already infected with the very pathogen should produce an unfavourable outcome. However, similar counterintuitive principle is followed in vaccine therapy or therapeutic vaccines, a type of antigen-specific immunotherapy, which has been in the limelight in recent years for the treatment of cancers, autoimmune and infectious diseases [32, 33]. Contrary to prophylactic vaccines that are used to prevent diseases, in vaccine therapy, the administration of the antigens of a pathogen to a patient, who is already infected with the very pathogen, helps in curing or alleviating the existing disease. Vaccine therapy or therapeutic vaccines work by reprogramming the host immunity by activating and boosting the antigen specific immune response, where the T-cell-mediated immunity is enhanced via stimulation of both CD8 (killer/cytotoxic) and CD4 (helper) T cells [32-34]. The humoral response is also promoted by stimulated (follicular helper) CD4 T cells that help the B cells in producing antibodies [32-34]. The vaccine therapy, in a way, justifies the rationale behind the 'law of similars'.

In the present context, it is essential that the modulated proteins of characteristic immunogenicity present in the homeopathic medicines are delivered to the antigen-presenting cells (like dendritic cells and macrophages) retaining their conformation and in effect, their characteristic immunogenicity. Fortunately, proteins can enter the system sublingually retaining their conformations [35]. It may be noted that the gut environment is not favourable for the delivery of the proteins, which are enzymatically degraded by the gastrointestinal fluids at a low pH [36].

4. How do Homeopathic Medicines Containing Microbial Lysates Mediate Non-specific Immunotherapy and Boost Antigen-specific Immunotherapy?

Microbial lysates containing mixtures of bacterial, fungal and viral lysates are immunomodulatory in nature as bacterial lysates are well-known as immunomodulators that show beneficial effects in the treatment of a wide variety of bacterial, fungal and viral diseases [37-39]. The immunomodulatory actions (non-specific immunotherapy) of microbial lysates arise from the TLR-PAMP interaction (PAMPs binding to TLRs), given that TLRs (Toll-like receptors) are a family of pattern recognition receptors expressed on the host antigen-presenting cells like dendritic cells and macrophages, whereas PAMPs (pathogen-associated molecular patterns) are conserved molecular structures of microbes like surface proteins, nucleic acids, polysaccharides, and peptidoglycans available in the microbial lysates [37-39]. Incidentally, non-specific immunotherapy mediated by microbial lysates can explain an enhanced immune response obtained from homeopathic dilutions of ethanol without any drug (blank dilution) in murine infection with Trypanosoma cruzi [40].

Importantly, for successful antigen-specific immunotherapy, immunostimulants and adjuvants are required along with the antigens. Microbial lysates present in the homeopathic medicines act as immunostimulants and flagellin, a bacterial protein and cyclic di-GMP, a bacterial messenger present in bacterial (microbial) lysates can act as mucosal adjuvants [41-43].

5. Experimental Support for the Hypothesis and Clarification of Some Issues

In conformity with the proposed hypothesis, it has been found that homeopathic medicines can activate macrophages, stimulate phagocytosis and modify antibody (immunoglobulin, Ig) production [44]. Also, homeopathic medicines have been reported to show immunomodulatory actions by altering the levels of cytokines like interferon-gamma (INF-γ), tumour necrosis factor-alpha (TNF-α) and interleukins (IL-1, IL-2, IL-4, IL-5, IL-6, IL-10 and IL-12), though the mechanism behind such actions remained unknown [44, 45]. Some other relevant issues in the context of the proposed hypothesis have been clarified below.

First, though the microbial incorporation in the homeopathic medicines during their preparation is expected to be low (Sec.2), an appreciable immune response is still expected from homeopathic medicines, given that the number of proteins in a single bacterial cell is ~ 10^6 [46] and antigen of the amount of ~ 10 ng [47] or even ~ 10 pg [48] can generate a significant immune response.

Second, given that around 2-3% of the airborne microbes are pathogenic [9, 10], it is expected that homeopathic medicines, as opposed to bacterial lysates, contain lysates of mostly non-pathogenic microbes (excluding a small group of homeopathic medicines called 'nosodes' [49] that additionally contain lysates of pathogenic microbes, as nosodes are prepared from diseased products containing pathogens). Incidentally, the lysates of non-pathogenic

microbes can be immunomodulatory in nature, as PAMPs (pathogen-associated molecular patterns) are present in all the microorganisms irrespective of their pathogenicity and are better understood as MAMPs (microbe-associated molecular patterns) [42, 50]. Indeed, non-pathogenic microorganisms (including probiotics) inactivated by heating or lysis have shown beneficial immunomodulatory actions via TLR-MAMP interaction [42, 50].

Third, a question may arise regarding the possibility of fluctuations in the quality of microbial lysates (effectively, in the quality of homeopathic medicines), given that the amounts and types of the ambient microbes may show temporal and spatial variation. Incidentally, pharma clean rooms where homeopathic medicines are prepared restrict the ambient microbial counts within a specified range and, interestingly, the nature and abundance of the microbes are almost the same in the cleanrooms across the globe as the cleanroom microbes are primarily associated with skin flakes shed by the cleanroom personnel [12]. Above all, in line with the characteristics of bacterial lysates [37-39], the immune response generated from the microbial lysates should be almost independent of the type of microbes incorporated and lysed in the homeopathic medicines, as the TLR ligands like proteins, lipoproteins, lipopolysaccharides, and peptidoglycans present in the microbial lysates, not the microbes per se, are responsible for the immunotherapy [37-39].

Fourth, among the different types of proteins present in the microbial lysates (bacteria contain ~ 10^3 types of proteins [22, 46]), bacterial surface layer proteins, outer membrane proteins, heat shock proteins, flagellin etc. show strong immunogenicity and some fungal and viral proteins are also highly immunogenic [21, 51, 52]. At this juncture, it is not possible to predict which specific type(s) of immunogenic proteins play(s) the key role in immunotherapy in the present context.

Fifth, proteins in the homeopathic medicines should be present in denatured and/or partially denatured state (denatured proteins are unfolded proteins) as ethanol, a component of the homeopathic drug vehicle, denatures proteins partially or completely depending on the concentration of alcohol and the characteristics of the proteins [53]. Incidentally, proteins have a tendency to form aggregates, especially in their denatured or partially denatured states [54]. Hence, the proteins in the homeopathic medicines should be present primarily as aggregates. In biopharmaceuticals, the protein aggregates can give rise to exaggerated immune response and can degrade the efficacy of the drugs due to the formation of antidrug antibodies (ADAs), whereas in vaccines, such aggregate-induced enhancement of immunogenicity may be useful, if properly regulated [54, 55]. At present it is difficult to predict whether the protein aggregates in homeopathic medicines enhance the immunogenicity of the medicines to the benefit of the patients.

Sixth, the term immunotherapy, as defined in the mainstream (and followed here) should be contrasted with homeopathic immunotherapy (also called isopathy or isotherapy) as coined by the homeopathic practitioners where homeopathic dilutions prepared from the causative agents like dust mites and pollens are employed to cure or palliate diseases like allergy and asthma [56].

6. Importance of Dilution and Dosage in Homeopathy from the Perspective of Immunotherapy

As discussed earlier (Sec.3), in vaccine therapy (and accordingly, in antigen-specific immunotherapy in homeopathy), T-cell mediated immunity plays the central role [32-34]. It has been found that low antigen doses generate T cells of high functional avidity (high antigen sensitivity), which can detect very low levels of antigens [57]. Also, low amounts of antigens provide high avidity memory T cells (both CD4 and CD8) and superior antibodies showing excellent affinity (antigen-antibody binding strength) [57]. However, very low doses of antigens may not elicit any useful response [57]. Hence, it is expected that the lower the dose of the antigens or equivalently, the higher the dilution (within certain limits) of a homeopathic medicine, the stronger the immune response is, which roughly agrees with the homeopathic 'law of infinitesimals' ('the more the dilution of a medicine, the more is its potency').

7. How do Ultra-diluted Medicines Lacking in Drugs Mediate Antigen-specific Immunotherapy?

In ultra-diluted homeopathic medicines (diluted beyond 'Avogadro's limit'), no drug is present to modulate the proteins available from microbial lysates formed during the preparation of ultra-diluted medicines. However, along with unmodulated proteins, ultra-diluted medicines should contain small amounts of modulated proteins from the portions of previous dilutions (lower dilutions where drugs are available to modulate the proteins), as serial dilution is used in homeopathy. Incidentally, protein molecules can interact among themselves through allosteric communication, given that allostery is a phenomenon where perturbations at the specific sites of protein (nonfibrous) molecules are transmitted through allosteric networks (networks of connected amino acid units or clusters of units) to the distant sites (that can extend over multiple protein-protein linkages) where the activity of the functional states is regulated [58, 59]. Allosteric signals can travel through protein networks containing water molecules [60].

As discussed earlier (Sec.5), in homeopathic medicines proteins are present mostly as aggregates and, given that proteins tend to self- as well as cross-associate [54, 61], the

protein aggregates in ultra-diluted medicines should contain both unmodulated and modulated protein molecules. Incidentally, protein molecules are not static entities and show thermal energy driven long-range correlated conformational motion or breathing vibration (low-frequency vibration of timescale ~ 1msec – 1sec [62]) arising from the presence of multiple substates in the energy landscapes of proteins [63]. It is known that coupled oscillators with comparable natural frequencies synchronize their vibration (self-synchronization) and the synchronization frequency of two coupled oscillators lies between their natural frequencies [64, 65]. In an ensemble of coupled oscillators, the synchronization frequency depends on the distribution of the natural frequencies of the oscillators and their coupling strength [64, 65]. Considering a simple scenario, it is expected that vibrating unmodulated protein molecules and modulated protein molecules of the same family (i.e., of similar conformation and hence, of comparable natural frequencies) that are allosterically coupled [66] would readily synchronize their vibrations. The synchronization frequency should be an intermediate one lying between the natural frequencies of the unmodulated and modulated protein molecules.

Interestingly, the attainment of an intermediate synchronization frequency of the protein molecules necessitates a concurrent attainment of an intermediate conformation of the protein molecules, i.e., a conformation intermediate between the conformations of the unmodulated and modulated protein molecules, because the conformations and the conformational vibrations [59, 63, 66] of protein molecules should be interrelated. Hence, after vibration synchronization, the unmodulated protein molecules should attain an intermediate conformation suggesting that even in the absence of drugs, the information of modulation (drug-induced conformational modulation) can be transmitted from the modulated proteins to the unmodulated proteins through protein-protein interactions. However, the resultant intermediate conformation of the protein molecules indicates that the modulation achieved through protein-protein interaction is intermediate (partial) with respect to that achieved through drug-protein interaction. Hence, by controlling the degree of dilution, the modulation and effectively, the immunogenicity of the proteins can be controlled. However, in extreme dilutions, the modulation of proteins may be insignificant to provide any useful antigen-specific immunotherapy.

8. Why are Clinical Trial Results in Homeopathy Inconsistent?

First, immunotherapy in homeopathy is accidental and not by design and hence, is in crude form. Accordingly, some inconsistencies in the clinical trial results are not surprising.

Second, in immunotherapy a group of non-responders (patients refractory to immunotherapy) normally exists, which may bias the findings of the clinical trials [67]. Also, heterogeneities in immunotherapy like variation in the immune status of the patients, size and frequency of the dose, administration routes of the immunogens, patients' age, sex, obesity, race and gut microbiome may distort the clinical trial results [67-70].

Third, some variations in the immune response may arise from the lack of control on the actual amount of dose delivered through sublingual route because of dilution of the immunogens by saliva and individual variation in the thickness and permeability of the sublingual epithelium [41, 71].

Fourth, as most of the patients seek homeopathic treatment for chronic pain, cough, asthma, irritable bowel syndrome, arthritis and other lingering illness, where placebo response is substantially high [72, 73], the clinical trial results in such cases may not reveal the true efficacy of the homeopathic medicines. Accordingly, enrichment strategies and adaptive designs for clinical trials may be implemented to get around the problems [74, 75].

9. Conclusions

Homeopathic therapy can be understood as a protein-based antigen-specific immunotherapy (albeit, in rudimentary form) akin to vaccine therapy, where the proteins are available from microbial lysates formed in the homeopathic medicines during their preparation. The proteins are conformationally and in effect, immunogenically modulated through drug-protein interactions and in ultra-diluted medicines through protein-protein interaction when no drug is available. The microbial lysates present in the homeopathic medicines also stimulate the immune response through their immunomodulatory effects (non-specific immunotherapy) and boost the antigen-specific immunotherapy by providing adjuvants. The reported immunomodulatory activities of homeopathic medicines support the proposed hypothesis. Also, the hypothesis, without invoking any contested concept, can explain the basic tenets of homeopathy and can resolve issues like the absence of drugs in ultra-diluted medicines and controversies related to clinical trials in homeopathy.

The hypothesis needs to be tested through rigorous experiments and the roles of other immunogenic microbial constituents present in the homeopathic medicines like protein fragments/peptides, peptidoglycans, lipopeptides, lipopolysaccharides, beta-glucans and nucleic acids should be studied from the viewpoint of immunotherapy. Finally, homeopathy should be probed and refined from the perspective of immunotherapy so that a simple, reliable and inexpensive immunotherapy may be developed for diverse diseases.

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Declaration of Competing Interest

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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